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Unmasking of latent herpesvirus infection

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UNMASKING OF LATENT HERPESVIRUS INFECTION

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Cervical specimens were collected from 116 pregnant women during 2 different pregnancy periods and from 12 non-pregnant women in attempts to isolate Herpesvirus hominis type II. None of these women had detectable herpetic lesions. Virus was isolated from 9 pregnant women and 2 from non-pregnant women. These results indicate that a larger number of women are shedding the virus asymptomatically. Serological correlation was made by the indirect hemagglutination (IHA) test. The IHA titer increased significantly only in those women from whom virus was isolated, but a moderate increase in titer was noted in a number of the women from whom virus could not be detected. The reason for this increase is not known.

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CHAPTER I

INTRODUCTION

Herpesviruses that can cause infections in man and animals include herpes simplex, herpes B, pseudorabies, varicella-zoster (V-Z), Epstein Barr virus (EBV) and cytomegalovirus. These viruses are grouped according to similarities in morphological, physicochemical, and biological properties. Some distinguishing characteristics of this group are that the virus capsid has a icosahedral shape, is composed of 162 cylindrical capsomeres and has a diameter of approximately 100-150 mu. Within this structure is contained the viral DNA. The viruses are susceptible to ether, heating (56°C) and pH below 3. Most members of the family have a special affinity for cells of ectodermal origin and tend to produce latent infection (34).

Around 100 A.D. the Roman physician 'Herodotus' first described the herpetic eruptions which appeared about the mouth at the crisis of simple fevers (45). About 166 years later, a French physician, Astruc, recorded herpes of the genital tract in both men and women (4). The term herpes had, however, been used since the earliest epoch

of Greek medicine to include spreading cutaneous lesions of varied etiology (14). By the 19th century, the generally accepted use of the term was restricted to certain diseases associated with vesicular eruptions (33). By the latter part of that century, a further distinction between infections caused by herpesviruses and by members of the pox group was made on the basis of cytopathological features (63).

Initially, diseases of the herpes group, which were characterized by the common finding of multinucleated giant cells and 'ballooning cells,' included herpes febrilis, herpes genitalis and herpes zoster, but by the early part of the 20th century, zoster had been well separated from the other two viruses on laboratory, clinical and epidemiological grounds (35). The term herpes simplex was used when viruses with similar biochemical and biophysical characteristics were isolated from a variety of animal species (3).

Gruter was the first investigator to find a laboratory tool for studying herpesviruses. His studies began around 1911 and were completed by 1914, but the report describing the successful transmission of herpetic ocular virus from man to rabbit cornea was not published until 1920 (23). The agent was demonstrated to be filterable by Luger and Landa (42) and was found by Doer and Schanable (17) to cause a paralytic fatal encephalitis in rabbits.

Lowenstein (41) showed that material from human herpetic infections other than of the eyes and from areas

such as skin and mucous membranes also produced ketatoconjunctivitis in the rabbit. The agent was even demonstrated in the saliva of asymptomatic patients (36).

Gruter (23) later established that passage of non-encephalitic strains in rabbit brain markedly increased their capacity to induce a fatal paralytic encephalitis.

Although herpetic infection of the male and female genital tract was described as early at 1736 (4) and its clinical features were well defined in the next century by French and German physicians (16), it was not until 1883 that Unna recorded histological observations from these genital infections (62). The concept of infectious virus was still unknown, and various hypotheses were offered as to the etiology, i.e., sexual neurasthenia, a secondary effect of other venereal diseases, and nervous skin diseases (38). Following the observation that the rabbit cornea was suspectible to herpetic infection, Baum (6) used this system to demonstrate similar effects with two genital specimens. Lipschutz (38) inoculated material from genital herpetic lesions into the skin of 31 humans and elicited a clinical infection within 48-72 hours in six cases and 24 days in one case. He was able to detect a difference between genital and non-genital materials by their clinical evolution and cytopathologic appearance in the rabbit cornea. He further demonstrated that the cornea previously infected with herpes febrilis virus remained

susceptible to later inoculation with venereal herpes material. On the other hand, the cornea was immune to re-infection with herpes febrilis virus.

Herpes simplex

Synonyms of herpes simplex include herpes simplex virus (HSV), Herpesvirus hominis (HVH) and herpesvirus. Recently it became apparent that isolates of this virus from human beings can be separated into two groups by antigenic and biological differences (57).

Herpesvirus hominis type I (HVH I) is associated with most non-genital infections, especially skin lesions above the waist, encephalitis, stomatitis, eye infections, and some cases of generalized herpes simplex. Type II (HVH II) is found primarily in and on the genitilia and surrounding areas, can be venereally transmitted, and usually is the cause of generalized infections of the newborn (57).

Infection with herpes genitalis (Type II) can produce illness of varying severity. The majority of infections in women do not produce symptoms of sufficient severity to warrant medical attention. In several surveys of women, about 40% of the infections were not associated with symptoms; lesions could not be detected by gynecological examination in about an equal percentage of infected women (29). Viral lesions are most commonly found on the vulva, vagina, cervix, and perineum. Less common sites are on the thighs, buttocks, and mons pubis. The chief symptoms associated with lesions

are hyperesthia, burning, itching, and pain on urination. Dyspareunia and burning leukorrhea are commonly experienced (57).

In the male, a vesicular eruption on the glans, prepuce or shaft of the penis is usually produced. In addition to the pain and irritation associated with lesions, the patient may also experience a scant watery urethral discharge and pain on micturition. Symptoms of urethritis and prostatis appear to occur in the absence of an overt lesion on the penis in some patients. Viruses have been isolated from cases of non-specific urethritis and prostatis, usually however from a minority of the patients studied (57).

Various techniques have been used to determine antigenic type and to differentiate type specific antisera to HVH, e.g., chorioallantoic membrane plaquing, kinetic neutralization, quantal microneutralization, complement fixation, etc. (21). In 1970 Fuccillo et. al. (21) reported that the levels of antibody to HVH I and HVH II detected by micro-indirect hemagglutination (IHA) were slightly higher than microneutralization test; therefore, separation of cross reacting antibodies should be easier to evaluate. With this technique the effects of recurrent episodes of herpesvirus infection on the levels of type specific antibody can be rapidly demonstrated.

Neonatal herpes is an infection of infants within the first four weeks of life. The infection is usually acquired at birth, and the source of virus is the infected birth

canal of the mother. Consequently, HVH II appears to be responsible for more than 80% of the cases. The severe disseminated infection may become evident at any time from birth to 24 days of age with most illness beginning from 3-14 days after birth. The signs accompanying the illness are not specific and may include poor feeding, lack of weight gain, vomitting, diarrhea, respiratory difficulty, hypoactivity, vasomotor instability, jaundice, conjunctivitis or neurologic abnormalities. Abnormalities of multiple organs may become manifest during the disseminated infection. About half of the cases showed evidence of involvement of the central nervous system. Survivors of the illness are often left with sequelae such as microcephaly, hydrocephaly, parenceph-alitic cysts, and varying degress of psychomotor retardation (57).

Josey et. al. (30) have recently reviewed evidence for the possible association of type II virus with cancer of the cervix. They have noted an increased incidence of cervical dysplasia, carcinoma in situ and invasive cancer in women detected to have herpetic genital infection. This association may not be the cause and effect since carcinoma of the cervix and genital herpes may only be related to common factors, e.g., promiscuity. In 1972, Frenkel et. al. (20) reported that a portion of HVH II DNA was found in cells of cervical carcinoma.

Herpesvirus hominis can also cause infection in various species of animals. The virus can cause infection in the

rabbit at almost every site inoculated, e.g., cornea, conjunctiva, skin, ovary, testes. Goodpasture and Teague (21) demonstrated that HVH could be transmitted from a peripheral infection directly to the central nervous system along sensory, motor or sympathetic nerve fibers, depending on the site of inoculation.

Plummer et. al. (52) and Nahmias et. al. (46) found differences in the virulence of HVH I and HVH II strains which depended upon the route of inoculation and the age of the rabbit inoculated. None of the adult rabbits developed paralysis after being infected with several HVH I strains by intramuscular or intradermal routes, while most of the adult rabbits infected with HVH II became paralyzed (52). However, in young rabbits (8 weeks of age), 9 out of 10 inoculated intramuscularly and 5 out of 10 intradermally with HVH I developed paralysis. Nahmias et. al. (46) found that in 88 adult rabbits HVH II caused twice as many deaths from encephalitis as HVH I, and intracerebral inoculation with HVH II killed two-thirds of the rabbits, whereas none of the animals died from a similar dose of HVH I.

Levaditi and Nicoulou (37) were able to infect directly the vagina and penis of rabbits with HVH of unrecorded origin. They demonstrated that genital transmission could occur, since males placed in contact with females with vaginal infections developed penile lesions.

There is a progressive increase in resistance in mice to HVH infection with age. Johnson (27) in 1964 demonstrated that 10 times more 'HFEM' virus given intracerebrally was required to kill young adults than newborns, whereas at least 1000 times more virus was needed to kill adult mice by extraneural inoculation (intraperitorneal, subcutaneous or intranasal). Chzhu-Shan (12) reported that a HVH I strain was more virulent by the intraveneous route and less virulent by the intradermal route than a HVH II strain. Plummer and Hacket (51) noted that intramuscular inoculation with HVH II cause death in a higher percentage of adult mice than did HVH I. Infection of the mouse penis could also be obtained with HVH II strains by either direct inoculation or by caging male mice with females with vaginal infections.

In 1974 Baker et. al. (5) demonstrated that 4 to 6 week old female Swiss mice inoculated intravaginally with HVH II developed vaginitis, posterior paralysis, encephalitis and death. They also found that BCG-immunization and treatment with HVH II antiserum could protect these mice from infection.

There was no difference in the effect of various strains of rats. Chzhu-Shan (12) reported that two day old rats were highly susceptible to HVH whether or not the mother had been infected previously.

Burnstein (10) reported that when hamsters were inoculated with HVH I by the genital route, half of the animals developed a fatal paralysis. He suggested that paralysis

and death were due to spread of virus by means of local neural route. Nahmias et. al. (46) indicated that after genital infection, virus is disseminated via the blood stream and that encephalitis can be demonstrated histologically. Fern and Low (19) found that intravenous infection of pregnant hamsters with HVH I caused adrenal lesions and, less commonly, lesions in the liver and placenta without killing the animals or affecting the offspring. On the other hand, HVH injected directly into 13-day old hamster fetuses caused death within 48 hours. Nahmias et. al. (46) inoculated over 800 newborn hamsters with varying dilutions of HVH I and HVH II strains by intraplaural, intraperitorneal or subcutaneous route. They found that higher concentrations of viruses of either type given by any route resulted in uniform mortality. Even with lower amounts of virus, no more than one-half of newborns survived depending on the route of inoculation.

Guinea pigs do not develop an encephilitis following corneal inoculation, and keratitis is milder than in rabbit. However encephalitis can be induced in the guinea pigs by intracerebral inoculation (7).

Blanz and Caminopetous (8) reported that several animals, e.g., sheep, chickens, pigeons, and toads, are resistant to HVH inoculated by a variety of routes.

Properties of Latent Infection

A latent infection is one in which the viral genome is present in the cell, but infectious virus can not be recovered, except during episodes of overt disease and occasionally during periods of fever, hormonal changes associated with menstrual cycles, emotional upsets, ingestion of oral contraceptive hormones, and pregnancy (54). A persistent infection on the other hand, is one in which infectious virus is continually released, even in the presence of circulating antibody and in the absence of symptoms of disease (54).

Goodpasture (22) in 1929 postulated that herpesviruses induced characteristic latent infections. He noted that infectious virus was neither released nor detected in patients suspected of having latent infections. However, certain changes in internal environment and certain nonspecific stimuli can trigger production of detectable virus. There are a number of evidences indicating that recurrent infection or recrudescent infection by herpesviruses is depended upon the depression of cell mediated immunity (CMI). For example, Lodmell et. al. (40) reported that the combined attack of stimulated leukocytes and antiviral antibody eliminates HVH infection in vitro. These investigators reasoned that the leukocytes exert a toxic effect upon some of the virus-infected cells, break intracellular bridges, and inhibit viral reproduction. Viral-infected cells not killed by the leukocytes are destroyed by the antiviral

antibody and complement before intracellular bridges are re-established. In 1974, Baker et. al. (5) reported that BCG immunization can protect 6 week old female Swiss mice from recurrent herpesvirus infection, suggesting that CMI mechanism may not be specific.

During pregnancy, evidence indicates that CMI is depressed at least partially based on impaired cutaneous and *in vitro* lymphocyte responses to PPD and prolonged homograft survival of skin grafts in pregnant women (60). The impaired CMI that is found in pregnant women, including immunosuppressed individuals, can be assessed by testing the response of their lymphoid cells *in vitro* to the mitogen phytohemagglutinin (PHA) (53). The reduction in maternal lymphocyte responses to PHA in pregnant women could result from the cumulative action of blocking antibodies, hormones, and certain plasma proteins (53). Various hormones, including chorionic gonadtropin, corticosteroids, estrogens, and progesterones, show immunosuppressive properties as measured by reduction of the lymphocyte responses to PHA or prolonged homograft survival (53).

Evidence that glycoprotein can reduce maternal CMI includes reports that a placenta glycoprotein and alpha-globulin impair lymphocyte responses to PHA. The fetal protein, alpha-fetoprotein and carcinoembryonic antigen, found in the serum of pregnant women, could also depress maternal CMI by coating lymphocyte antigen receptor sites (61).

Depressed maternal CMI during pregnancy benefits the conceptus, but increases maternal risk to certain infections and to malignancy (18). In 1967 Peters (47) reported an 11% five year survival in women developing breast carcinoma in the last half of pregnancy, compared with 48% survival in women developing breast carcinoma in the first half of pregnancy, puerpurium, or in non-pregnant women.

In 1972 Smith et. al. (60) reported that lymphocyte reactivity to PPD diminished as pregnancy advanced and was restored to a normal level during the first ten days or so of the puerpurium. These changes are associated with a corresponding rise in the level of lymphocyte depressing factor (LDF) in the serum, which falls again after delivery. Diminished lymphocyte response to PPD is not due to sensitized cells having been removed from the circulation.

In 1973 Anderson et. al. (1) reported that after patients with frequent recurrent Herpesvirus hominis infections were immunized with BCG, all had a decrease frequency of recurrent infections. The patients who became pregnant after vaccination were unable to elicit DH response to PPD and showed more recurrent lesions than the non-pregnant vaccinees.

The object of this study was to determine if asymptomatic pregnant women shed HVH by collecting cervical specimens during the first and second trimester. The indirect hemagglutination test (IHA) was used to correlate discovery of virus to antibody response against HVH I and HVH II.

CHAPTER II

MATERIALS AND METHODS

Antibiotics

Penicillin and Streptomycin. A stock solution containing 20,000 units potassium penicillin G (Chas. Pfizer and Co. New York) per milliliter of sterile deionized distilled water (DDW) was stored at -20°C. Five ml of this solution was added to each liter of medium giving a concentration of 100 units penicillin and 100 ug streptomycin per ml of medium.

Amphotericin B. Powdered amphotericin B (Fungizone) obtained from E. R. Squibbs and Sons, New York was dissolved in Hank's balanced salt solution (HBSS) at a concentration of 12,000 ug/ml and stored at -20°C. For use, 0.4 ml was added to 1 liter of medium to give a concentration of 4.8 µg amphotericin B per ml.

Solutions and Media

Hank's Balanced Salt Solution (HBSS). Ten times concentrated solutions of HBSS were prepared by dissolving 49.5 gms of dehydrated HBSS (GIBCO) in 5 liters of DDW. This solution was filter sterilized, dispensed into 8 oz.

prescription bottles, and stored at -20°C. Single strength HBSS was prepared by adding 10 ml of the 10 x stock solution and 0.5 ml of 7.5% $NaHCO_3$ to 89.5 ml of DDW.

Earle's Balanced Salt Solution (EBSS). This solution was purchased in powdered form from GIBCO. Ten times concentrated stock solutions were prepared by dissolving 43.5 gms of the EBSS powder in 5 liters of DDW. After proper mixing, the solution was sterilized by filtration. To prepare single strength EBSS, 10 ml of the 10 x stock solution and 3 ml of 7.5% $NaHCO_3$ were aseptically added to 87 ml of sterile DDW.

Phosphate Buffered Saline (PBS). This solution was prepared as follows:

Solution A

Components	Amounts
NaCl	16.00 g
KCl	0.40 g
Na_2HPO_4	2.30 g
KH_2PO_4	0.40 g
DDW	1,500 ml

Solution B

$MgCl_2$	0.20 g
DDW	200 ml

Solution C

$CaCl_2$	0.20 g
DDW	200 ml

These solutions were autoclaved for 20 min at 15 pounds/ square inch. After cooling to 5°C, the PBS was prepared by adding solution B and then solution C to solution A, and then adjusting the volume to 2 liters with sterile DDW. The PBS was dispensed into sterile 8 oz. prescription bottles and stored at room temperature.

Pd. A modification of PBS was parpared by dissolving the components of solution A in 2 liters of DDW. This solution was dispensed in 8 oz. prescription bottles and autoclaved at 15 psi. It was referred to as Pd and was used as a balanced salt solution. The advantage of Pd over other balanced salt solutions was the absence of Mg and Ca ions. This facilitated trypsinizing procedures and the action of versene.

Trypsin-versene Solution. The trypsin-versene solution was prepared in the following manner:

Components	Amounts gms/1 DDW
NaCl	8.0
KCl	0.4
$NaHCO_3$	0.58
glucose	1.0
trypsin	1.0
versene	0.2

All the ingredients except versene and $NaHCO_3$ were dissolved in the DDW and stirred for 1 hour. Then the

versene and $NaHCO_3$ were added. The solution was filtered through a Seitz-filter, dispensed into test tubes and stored at 4°C. This solution was used for trypsinizing tissue to establish primary cell cultures.

Fetal Calf Serum. Fetal calf serum was purchased from GIBCO. Serum was heat inactivated at 56°C for 30 min shortly before it was added into the medium.

Patient's Serum. Five mls of blood were drawn from each patient, allowed to clot, and centrifuged to remove the serum. The sera were stored at -20°C. Prior to testing sera were heat inactivated at 56°C for 30 min.

Clinical Specimens for Virus Isolation. Specimens were taken from patients' cervixes by a gynecologist and put directly into the transport media.

Transport Medium. The media used to transport the clinical specimens was medium #199 with 10% FCS and gentocin at a concentration of 0.1 ml/100 ml of medium. Two ml aliquots of this medium were dispensed into sterile screw cap tubes.

Tannic Acid Solution. A 1:1000 stock tannic acid solution was prepared by dissolving 1 gm of tannic acid in 1 liter of DDW. Before use this stock solution was diluted 1:40,000 with PBS.

1% and 2% Normal Rabbit Serum. One and two percent of normal rabbit serum (NRS) were prepared by adding 1 and 2 ml of heat inactivated NRS to 99 and 98 ml of PBS respectively.

Medium #199. Medium #199 was purchased from GIBCO as a powder. The liquid medium was prepared in the following manner:

Order of Addition	Components
1	600 ml DDW
2	9.9 gm of M #199 powder
3	2.2 gm $NaHCO_3$
4	5.0 ml pen-strep
5	DDW to a total volume of 1000 ml
6	100 ml FCS

After thoroughly mixing, the M #199 medium was sterilized by filtration through a millipore filter and stored at 4°C until use.

Procedure for Passing of Monolayer Cells. The monolayer was washed 3 times with approximately 40 ml of Pd. Five ml of trypsin solution was added to the monolayer and it was incubated at 37°C for 10 min. Then 10 ml of medium #199 was added to the flask and mixed gently 3 times using a 'suckling-blowing' action. The trypsinized cell suspension was transferred into a conical centrifuge tube and centrifuged for 5 min at 900 rpm. The supernatant was decanted and the cells were gently resuspended in 4 ml of medium #199

and transferred to 16 oz prescription bottles (approximately 20×10^5 cells per bottle).

Procedure for Infecting the Cells. A 1 ml aliquot of the clinical specimen was added to a confluence monolayer of Vero cells in a Falcon plastic flask (#3012), incubated at 37°C and observed for the presence of cytopathic effect (CPE) at 24 and 48 hours. The culture flasks were incubated for 5 days and then if no CPE had occurred they were reported as negative.

Preparation of Virus Pools. Strain KOS I of HVH I and strain 196 of HVH II were obtained from W.E. Rawls, Baylor University College of Medicine, Houston, Texas. Virus pools were prepared by infecting confluent monolayers of Vero cells. After 24 hours of incubation at 37°C, the cultures were subjected to three rapid freeze-thaw cycles in an ethanol-dry ice bath and then centrifuged at 2500 g for 30 min. The supernatant fluid was dispensed in ampoules, frozen, and stored at -70°C until needed. Virus pools were titrated in Vero cells and contained from 4×10^5 to 2×10^6 mean tissue culture infective doses ($TCID_{50}$)/ml.

Indirect Hemagglutination (IHA). Sheep erythrocytes (a 3% suspension) were washed three times in PBS and then treated with an equal volume of freshly prepared tannic acid diluted 1:40,000 in PBS. The suspension was incubated at 37°C in a water bath for 15 min, then centrifuged at 600 g

for 10 min. After washing once in one volume of PBS the tanned cells were sedimented by centrifugation at 600 g for 10 min, the supernatant fluid was discarded, and the cells resuspended to a 3% concentration in saline.

Cells were then sensitized with a previously determined dilution of antigen by mixing equal volume of cells (3%) and antigen in saline (pH 6.4), and allowing the mixture to stand at room temperature for 15 min. The suspension was centrifuged at 600 g for 10 min (4°C) and the packed cells were then washed in two volumes of 1% NRS. Cells were then centrifuged and resuspended in 1% NRS to a concentration of 1%.

Tests were performed by the microtiter method utilizing a microdiluter and V bottom, soft, plastic plates (Cooke Engineering Co., Alexandria, Va.). Equal volumes (0.025 ml) of each reagent were used. After making two-fold dilutions in 2% NRS, 0.025 ml of 2% NRS and 0.025 ml of sensitized 1% sheep erythrocyte were added to each well. Plates were then sealed with cellophane tape, shaken by hand and read after incubation at room temperature for 1½ hr.

A known positive antiserum was included as a control in all tests. Other controls consisted of serum diluent incubated with sensitized and antigen-free tanned red cells.

CHAPTER III

RESULTS

All 69 pregnant women listed in Table 1 and placed in group 1 had low IHA antibody titers, the maximum being 128. Cytomegalovirus was isolated from patient #17 but the IHA response to HVH I and HVH II remained low. Group 2 patients, of which there were 37, those patients from whom no virus could be recovered but who had IHA antibody titers higher than the patients in group 1. The maximum titer in this group was 512, and the majority had titers of 256.

In Table 1 and placed in group 3 were patients #108 to 112 who were positive for HVH in the first culture but not in the second culture. The IHA antibody titers to HVH II were very high and remained so throughout the second trimester. The IHA antibody titers to HVH I of patient #111 remained elevated during both periods but those of 109, 110, 112 dropped considerably when retested in the second trimester. Patients #113 to 116 were positive for HVH in the second culture but not in the first. The IHA antibody responses to HVH II were maximal titer 4096 and remained so throughout the second trimester. The IHA antibody titers to HVH I of patients #113, 116 were very low through both sampling

periods. The titers of patients #114, 115 dropped considerably when retested in the second trimester.

It is interesting to note that virus isolation appears to coincide with maximal IHA antibody titers to HVH II in Table 2. For example, 12 females who were not pregnant and had no demonstrable lesions were included in the study and 2 of these women were found to be shedding HVH II asymptomatically (Table 2, group 3). Their IHA antibody titers of 2 blood samples taken at a 2 month interval remained elevated for the HVH II virus. The titer to type 1 virus was elevated in patient #11 but not in patient #12. However, the titer of the former patient dropped considerably in the second sample. The titer of patient #12 was low in the first sample and remained so.

All 7 women in Table 2 and placed in group 1 were negative for virus isolation and had low maximal IHA antibody titers of 64 or less. Among the 3 patients in group 2 from whom no virus could be recovered, all had a moderate IHA antibody response to both HVH I and HVH II antigens.

To correlate further the results obtained on the IHA antibody response of these patients, 26 students who reported to the University of Montana Health Service due to suspected herpesvirus lesions were included in the survey. As recorded in Table 3 no virus could be recovered from the lesions including 2 cervical swabs of students 1 to 9. The IHA titer

of students #2 and 9 measured low or moderate respectively to HVH II and high to HVH I. The lesions may not have been associated with HVH II.

Virus was recovered from the remaining students who all processed maximum HVH antibodies based on the IHA assay. The titer to HVH I was also high in students #11, 14, 15, 19, 21 and moderate in studnet #18. All the serum from the others were low against HVH I.

Table 1

Isolation of HVH during the first and second trimester of pregnancy. The sera were collected during the first trimester to determine the IHA antibody titer to HVH I and HVH II. The sera were collected later during the second trimester for the positive HVH isolation.

No.	Code	First Culture	Second Culture	IHA titer for HVH I	IHA titer for HVH II
Group 1					
1	M A	-	-	64	64
2	L A	-	-	32	32
3	J B	-	-	2	2
4	B	-	-	2	8
5	L B	-	-	8	8
6	K B	-	-	2	8
7	B B	-	-	2	2
8	D B	-	-	4	16
9	MAB	-	-	32	32
10	D B	-	-	4	16
11	P C	-	-	16	32
12	J C	-	-	16	16
13	N D	-	-	16	16
14	A D	-	-	4	2
15	J D	-	-	64	64
16	LDL	-	-	64	64
17	A F*	CMV		128	128
18	H G	-	-	64	32
19	J H	-	-	64	128
20	M H	-	-	32	32
21	C H	-	-	4	16
22	E H	-	-	2	4
23	D H	-	-	2	4
24	S H	-	-	8	8
25	A H	-	-	2	2
26	M H	-	-	2	2
27	P H	-	-	16	128
28	J H	-	-	8	8
29	B H	-	-	2	8
30	M H	-	-	4	8
31	B H	-	-	64	128
32	K H	-	-	2	2
33	B H	-	-	8	8
34	R J	-	-	4	4
35	C K	-	-	2	32
36	D K	-	-	2	2
37	B S	-	-	32	32

Table 1 cont'd.

No.	Code	First Culture	Second Culture	IHA titer for HVH I	IHA titer for HVH II
38	V K*	-	-	2	2
39	MAK	-	-	16	32
40	T K	-	-	2	4
41	S L	-	-	8	8
42	M L	-	-	4	8
43	KMLV	-		32	32
44	C M	-	-	16	64
45	CMG*	-		4	8
46	K M	-	-	64	8
47	M M	-	-	64	64
48	S M	-		16	16
49	V M	-	-	8	16
50	KME	-	-	2	2
51	A M	-		32	32
52	K P	-		4	8
53	A P	-		4	4
54	C P	-		64	128
55	A L	-	-	128	128
56	C S	-	-	16	4
57	JAS	-	-	32	32
58	B S	-	-	64	64
59	E S	-	-	64	64
60	D S	-		64	64
61	A S	-	-	4	128
62	K S	-	-	32	32
63	M S	-	-	32	32
64	R T	-		4	2
65	S T	-		64	64
66	K T	-		64	64
67	C W*	-	-	32	32
68	T W	-		64	64
69	M Y	-	-	8	8
Group 2					
70	L A	-	-	256	64
71	K A	-	-	256	256
72	K A	-	-	256	256
73	P B	-	-	256	256
74	M C	-	-	64	256
75	M D	-	-	512	8
76	N D	-	-	256	256
77	G D	-	-	256	256
78	M D	-	-	256	256
79	D F	-	-	256	256
80	C H	-	-	32	256

Table 1 cont'd.

No.	Code	First Culture	Second Culture	IHA titer for HVH I	IHA titer for HVH II
81	S H	-	-	8	256
82	K H	-	-	256	8
83	MJH	-	-	256	256
84	MJJ	-	-	256	16
85	T J	-		256	64
86	L K	-		256	2
87	S L	-		32	256
88	LLF	-	-	256	64
89	P M*	-		256	256
90	V M	-	-	256	256
91	JMK	-	-	256	32
92	A M	-	-	256	256
93	AMC	-		256	256
94	P O	-	-	256	256
95	K O	-	-	256	256
96	B P	-	-	256	256
97	S S	-	-	256	256
98	K S	-		256	256
99	J S	-	-	32	256
100	I T	-		256	256
101	M T	-	-	256	256
102	G W	-		256	32
103	N W	-	-	256	256
104	S W	-	-	256	256
105	R W	-		256	256
106	K Y	-		256	256
107	AYF	-	-	256	256
Group 3					
108	L B	HVH		4	4096
			-	4	4096
109	L G	HVH		512	4096
			-	8	4096
110	T S	HVH		4096	4096
			-	4	4096
111	S S	HVH		2048	4096
			-	2048	4096
112	O V	HVH		1024	4096
			-	4	4096
113	C A	-		2	32
			HVH	2	4096
114	M B	-		2048	4096
			HVH	4	4096
115	J C	-		512	4096
			HVH	8	4096
116	B H	-		2	4096
			HVH	4	4096

*patient moved

Table 2

Isolation of HVH from the non-pregnant women without detectable lesions. Sera were collected to determine the IHA antibody titers to HVH I and HVH II. Sera were collected later for the positive isolation.

No.	Code	Culture Result	IHA titer for HVH I	IHA titer for HVH II
Group 1				
1	K H	-	8	8
2	J L	-	8	8
3	N Y	-	8	8
4	B C	-	16	16
5	R J	-	32	32
6	M R	-	8	16
7	V W	-	32	64
Group 2				
8	D H	-	256	256
9	M M	-	256	256
10	W S	-	256	256
Group 3				
11	C M	HVH	256	4096
			2	4096
12	C T	HVH	8	4096
			8	4096

Table 3

Isolation of HVH from the lestions of the progenitalis areas of University of Montana students. Sera were collected to determine the IHA antibody titers to HVH I and HVH II.

No.	Code	Source of Specimen	Culture Result	IHA titer for HVH I	IHA titer for HVH II
1	C C	progenitalis	-	4096	4096
2	KMC	progenitalis	-		
		cervix	-	2048	16
3	K M	progenitalis	-	1024	4096
4	M L	progenitalis	-	4096	4096
5	W B	progenitalis	-	4096	4096
6	R L	progenitalis	-	64	4096
7	J N	progenitalis	-	8	4096
8	D P	progenitalis	-	4096	2048
9	MCM	cervix	-	2048	512
10	B M	progenitalis	HVH	4	4096
11	C C	progenitalis	HVH	4096	4096
12	D L	progenitalis	HVH	16	4096
13	J L	cervix	HVH	8	4096
14	O D	progenitalis	HVH	4096	4096
15	R P	progenitalis	HVH	4096	4096
16	C S	progenitalis	HVH	8	4096
17	E H	progenitalis	HVH	128	4096
18	D T	progenitalis	HVH	512	4096
19	G A	cervix	HVH	4096	4096
20	MRG	progenitalis	HVH	4	4096
21	S T	progenitalis	HVH	4096	4096
22	W W	progenitalis	HVH	2	4096
23	R V	progenitalis	HVH	2	4096
24	M L	progenitalis	HVH	64	4096
25	S L	progenitalis	HVH	8	4096
26	S D	progenitalis	HVH	16	4096

CHAPTER IV

DISCUSSION

Burnett et. al. (9) in 1939 showed that primary infection with herpesvirus normally takes place during the early childhood years. The virus remains latent, presumably in the cells at or near the site of primary infection, i.e., in the buccal mucosa or in the circumoral skin or in the spinal ganglia. It is probable that once infection has occurred the viral genome persists in the cells throughout the life of the individual. The precise mechanism for the unmasking of the latent virus is not known, but following certain stimuli such as infections by other agents, exposure to excessive light, stress, hormonal changes during menstruation, pregnancy, etc., the virus is activated. In most but not all individuals the familiar lesions develop.

According to Nahmias et. al. (46) recurrent infection is the asymptomatic viral reactivation and recrudescent infection is the recurrence of specific clinical lesions. Therefore, the patients with recurrent venereal herpetic lesions are classified as having recrudescent infection. Rasmussen et. al. (55) have demonstrated that interferon production in vitro by patients' lymphocytes in response to HVH antigen wanes some weeks after a recrudescent infection.

These investigators suggested that the waning of the interferon response may account for the cyclic development of recrudescent infections.

The presence of a 'typical herpetic' lesion can be misleading. In the studies conducted in this laboratory, no evidence could be found in some individuals of an association of lesions with the presence of any virus indicating that herpesvirus can replicate in the absence of a lesion.

For example, the pregnant women in this study had no history of HVH infection based on the recognition of an ulcerated lesion by either the patients themselves or the physician. Nevertheless, at least 9 of them were shedding HVH without any macroscopically detectable lesion during the first and/or second trimester. The indirect hemagglutination (IHA) antibody titers to HVH II were highly elevated at the time when viruses were isolated, but the titers to HVH I were highly elevated in only some of the patients. When sera were collected later, the titers to HVH II remained unchanged, but the titers to HVH I in several patients dropped markedly. The reason for this change in antibody titers to HVH I but not to HVH II may be due to the fact that HVH I was not replicating and, therefore, not providing an antigenic stimulus. The antibody titers against HVH I too dropped dramatically when measured during the second trimester. Active momentary replication of HVH I in some patients during the first trimester along with HVH II could have accounted

for the highly elevated IHA antibody titers against HVH I. Since HVH I isolation attempts were not made, this cannot be substantiated. Although HVH II could not be isolated in some patients during the first or the second trimester, sufficient antigen was apparently available to maintain the continued antibody response that was measured.

The disappearance or absence of the virus in patients with high antibody level may be due to the time of sampling, the technique of collecting specimens or to the activity of humoral antibody and CMI as reported by Lodmell et. al. (40). These investigators found that the combined attack of stimulated leukocytes and antiviral antibody eliminate the HVH infection in vitro. These investigators reasoned that the leukocytes exert a toxic effect upon some of the virus infected cells, break intracellular bridges, and inhibit viral reproduction. Viral infected cells not killed by the leukocytes are destroyed by the antiviral antibody and complement before intracellular bridges are re-established. The antibody also neutralizes all extracellular virus.

The highly elevated IHA antibody titers to HVH II during two testing periods in non-pregnant women may indicate that these women had persistent infections, since the time interval between the first and second testing periods was about 6 months. If they did not have persistent infections, one might assume that the IHA antibody titers against HVH II would have dropped in the second testing period. Unfortunately, the cervical specimens were not repeated by the time

the second serum was taken, as perhaps these women may have been found to be shedding HVH II from either the vagina or cervix.

The patients who were shedding HVH II with detectable lesions had very high IHA antibody titers against HVH II, but virus was not detected in 9 others who had a 'typical' herpetic lesion, of these 8 had high IHA titers to HVH II. The absence of virus was probably due to the reasons given earlier, but in 2 patients the lesions may not have been related to HVH II virus. Other causes as allergy or bacterial infection may have been responsible for the lesion since the IHA antibody titers was not elevated as compared to all the other students. However, according to the interviews made in this study, all of these patients had history of both HVH I and HVH II infections. The interview did not reveal any prevailing reasons for appearance of lesions in these students, but all indicated some type of stress stimuli, notably the academic pressure.

According to the literature, HVH infection is considered second to gonorrhea as a venereal disease problem. However, according to statistical data being gathered in this laboratory and a Newsletter from the University of Washington, HVH II infections are surpassing gonorrhea infections as the primary venereal disease seen in student health clinics. There may be as many as 5 HVH infections for every case of gonorrhea treated. In any case, the results infer that a a significantly larger number of women may be shedding the

virus during pregnancy than realized, and this in turn would increase the possible incidence of congenital infection. Newborn infants who contract the virus during delivery may produce a wide spectrum of symptoms ranging from mild central nervous system damage to fatal meningoencephalitis, jaundice, hypoactivity. Survivors of the illness are often left with microcephaly, hydrocephaly, parencephalitic cysts, and varying degrees of psychomotor retardation. In most cases, the infection is not recognized until the infant is acutely ill. In most cases, by the time the illness is clinically evident it is too late to treat effectively. Therefore, a method is needed to screen and detect very early HVH infections in newborns, e.g., screen the mothers' cord blood as well as any jaundiced newborn. Perhaps screening of all newborns with any type of vague illness would be advisable.

Although many infants born of mothers who have active viral infections appear normal, these infants should be observed for any clinical signs of the HVH infection for at least one month. The babies may still be shedding virus similar to congenital infections with measles virus and cytomegalovirus. Infected babies may shed these latter two viruses for up to 2 years after birth. Little is known about this aspect of congenital infections with HVH.

Even in the cases where a woman has a clinically evident infection as a lesion, only about 70% of the infants born to these women contract the infection. This fortunate

failure to contract the disease perhaps is due to various reasons: 1) the titer of virus in the birth canal may have been low, 2) bursting of the amniotic sac could have 'washed' the extracellular virus from the birth canal, 3) the specific antibody level of the mother was sufficiently high to enable rapid neutralization of the virus, or 4) amniotic fluid itself may have 'antiviral' properties.

Further consideration must be given to HVH infections in view of the implications of an association between HVH II and carcinoma of the cervix. Antibodies to HVH II are higher in the patients with carcinoma of the cervix than in the normal control populations (11). Therefore, it is suggested that women who have had HVH II infection have more frequent Pap smears.

Currently several methods are available to evaluate the states of latency and viral replication in pregnant women. Isolation of virus can be attempted when parturition is anticipated for at least 4 days. If delivery is expected much sooner, the immunoperoxidase method can be used to detect rapidly virus in cells taken from the cervix, vagina or a lesion. In order to protect the baby from infection, the mother and physician may elect to deliver by Caesarean section.

CHAPTER V

SUMMARY

Cervical specimens were collected from 116 pregnant women during 2 different pregnancy periods and from 12 non-pregnant women in attempts to isolate Herpesvirus hominis type II. None of these women had detectable herpetic lesions. Virus was recorded from 9 pregnant women and 2 from non-pregnant women. These results indicate that a larger number of women is shedding the virus asymptomatically. Serological correlation was made by the IHA test. The IHA titers increased significantly only in those women from whom virus was isolated, but a moderate increase in titer was noted in a number of the women from whom virus could not be detected. The reason for this increase is not known.

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